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Rat Ovarian Prostaglandin Endoperoxide Synthase-1 and -2: Periovulatory Expression of Granulosa Cell-Based Interleukin-1-Dependent Enzymes*

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ABSTRACT

This laboratory has previously shown that interleukin-1 (IL-1), a putative intermediary in the ovulatory process, is capable of up-regulating PG biosynthesis by cultured whole ovarian dispersates from immature rats. In part, this phenomenon was attributable to the stimulation of ovarian phospholipase A_2 activity. In this communication we examine the possibility that the PG-promoting property of IL-1 is also due to the up-regulation of PG endoperoxide synthase (PGS), the rate-limiting step in prostanoid biosynthesis. The *in vivo* expression of ovarian PGS-2 transcripts in the course of a simulated estrous cycle rose abruptly to a peak (35-fold increase over the control value; $P < 0.05$) 8–12 h after hCG administration (*i.e.* before or during projected ovulation). PGS-1 transcripts, in turn, were not significantly altered during the periovulatory period. Treatment of cultured whole ovarian dispersates with IL-1β resulted in dose- and time-dependent up-regulation of PGS-2 transcripts (as well as of immunoreactive PGS-2 protein and PGE_2 accumulation), characterized by an ED_{50} of 2 ng/ml and a maximal (72-fold) increase at 10 ng/ml. Although treatment with IL-1β also led to an increase in PGS-1 transcripts and immunoreactive PGS-1 protein, the relative magnitude of the effect was markedly reduced compared with that of PGS-2. Cotreatment with an IL-1 receptor antagonist completely reversed the IL-1 effects, thereby suggesting mediation via the IL-1 receptor. The ability of IL-1 to up-regulate PGS-2 transcripts proved relatively specific, in that other cellular regulators (insulin-like growth factor I, activin A, endothelin-1, transforming growth factor-α, tumor necrosis factor-α, vascular endothelial growth factor, leukemia inhibitor factor, hepatocyte growth factor, or keratinocyte growth factor) were not effective. The optimal IL-1 effect required heterologous contact-dependent coculturing of granulosa and thecal-interstitial cells. Taken together, these observations 1) reaffirm (by molecular probing) the granulosa cell as the primary site of ovarian PGS-1 and PGS-2 expression, 2) document an increase in ovarian PGS-2 transcripts before ovulation, and 3) reveal a marked dependence of ovarian PGS ($2 \gg 1$) transcripts, proteins, and activity on IL-1. The effects of IL-1 proved relatively specific, contingent upon somatic cell-cell cooperation, dose and time dependent, and IL-1 receptor mediated. These results are compatible with the proposition that the PG-promoting property of IL-1 is due, in large measure, to the activation of ovarian PGS transcription and translation. The ability of IL-1 to up-regulate ovarian PGS, an obligatory component of ovulation, is in keeping with the idea that IL-1 may constitute an intermediary in the ovulatory process. (*Endocrinology* **139:** 2501–2508, 1998)

A GROWING body of direct and indirect evidence supports the idea that intraovarian interleukin-1β (IL-1β) constitutes an intermediary in the ovulatory process (1). First, the *ex vivo* provision of IL-1β has been shown to bring about ovulation and to synergize with LH in this regard (2, 3). Second, the addition of an IL-1 receptor antagonist attenuates LH-supported ovulation under both *ex vivo* (4) and *in vivo* (5) circumstances. Third, some components of the intraovarian IL-1 system (*e.g.* IL-1β and the type I IL-1 receptor) appear to be expressed *in vivo* only during a narrow periovulatory window (6–9). Fourth, IL-1β induces a host of ovulation-associated phenomena such as the stimulation of ovarian hyaluronic acid biosynthesis (10), the induction of ovarian collagenase activity (11), the perturbation of ovarian plasminogen activation (12, 13), and the activation of ovarian nitric oxide synthase activity (14–16).

Yet another corollary of ovulation is the biosynthesis of PG, a phenomenon first suggested by Kuehl *et al.* (17). This periovulatory gonadotropin-driven event is due in part to the promotion of prostaglandin endoperoxide synthase (PGS) activity (18–20). Indeed, pharmacological (21–24) or genetic (25) inhibition/elimination of PGS activity has been reproducibly shown to arrest follicular rupture. Although the precise role of PG in the ovulatory cascade remains uncertain, it is highly likely that PG may serve as coordinating messengers for a series of ovulation-associated phenomena such as the induction of periovulatory

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hyperemia (26, 27) and the promotion of collagenolysis (28, 29).

As IL-1β is capable of promoting ovarian PG biosynthesis (30–33) and appears to be gonadotropin dependent (6), we examined the effect of treatment with IL-1β on the expression and translation of PGS-1 and PGS-2 by cultured whole ovarian dispersates of immature rat origin. We further undertook to establish the cellular localization, cyclic variation, and hormonal regulation of PGS.

Materials and Methods

Animals

Immature Sprague-Dawley female rats from Zivic-Miller Laboratories (Zelienople, PA) were killed by CO_2 asphyxiation on day 25 of life. The project was approved by the institutional animal care and use committee.

Hormones and reagents

Recombinant human IL-1β (2×10^7 U/mg) was provided by Drs. Errol B. De Souza and C. E. Newton, DuPont-Merck Pharmaceutical Co. (Wilmington, DE). A recombinantly expressed preparation of the naturally occurring human IL-1 receptor antagonist (IL-1RA) was provided by Dr. Daniel E. Tracey, Upjohn Co. (Kalamazoo, MI). PMSG was obtained from Sigma Chemical Co. (St. Louis, MO). Highly purified human CG (hCG; CR-127; 14,900 IU/mg) was supplied by Dr. R. E. Canfield through the Center for Population Research, NICHHD, NIH (Bethesda, MD). Insulin-like growth factor I (IGF-I) was obtained from Bachem (Torrance, CA). Endothelin-1 (ET-1) was purchased from Peninsula Laboratories (Belmont, CA). Recombinant human tumor necrosis factor (TNFα; 5×10^7 U/mg) was obtained from Genentech (South San Francisco, CA). Vascular endothelial growth factor, leukemia inhibitor factor, and keratinocyte growth factor were purchased from Pepro Tech (Rocky Hill, NJ). Transforming growth factor-α (TGFα) was obtained from Oncogene Science (Uniondale, NY). Activin A was contributed by Jennie P. Mather, Genentech.

McCoy's 5a medium (serum-free), penicillin-streptomycin solution, L-glutamine, trypan blue stain, and BSA were purchased from Life Technologies (Grand Island, NY). Collagenase (*Clostridium histolyticum*; CLS type I; 144 U/mg) was obtained from Worthington Biochemical Corp. (Freehold, NJ). Deoxyribonuclease (bovine pancreas), aminoguanidine hemisulfate salt (AG), PGE_2, $PGF_{2\alpha}$, diethyldithiocarbamic acid, *n*-octyl-β-D-glucopyranoside (octyl glucoside), and ribonuclease A (RNase A) were obtained from Sigma Chemical Co. T7 and SP6 RNA polymerases, pGEM7Zf$^+$, and other molecular biology grade reagents were purchased from Promega (Madison, WI). Nitrocellulose filters (0.45 μm) were obtained from Schleicher and Schuell (Keene, NH), LC rainbow mol wt markers were purchased from Amersham (Arlington Heights, IL), [^{125}I]Protein A was obtained from ICN Biochemicals (Costa Mesa, CA), and [^{32}P]UTP was obtained from New England Nuclear (Boston, MA).

Tissue culture procedures

Whole ovarian dispersates were prepared and cultured as previously described (34). In some experiments, isolated granulosa cells or highly purified thecal-interstitial cells from immature hypophysectomized rats were also used. The derivation and maintenance of granulosa and thecal-interstitial cells conformed to previously described methods (35, 36).

PGE_2 RIA

The RIA for PGE_2 was carried out as previously described (30).

Nucleic acid probes

The rat PGS-1 and PGS-2 complementary DNAs (cDNAs) (37) were generously provided in Bluescript vectors by Drs. Daniel Hwang and Shuenn S. Liou of Pennington Biochemical Research Center, Louisiana State University (Baton Rouge, LA). A 354 *Cla*I-*Eco*RI fragment of the original PGS-1 cDNA was subcloned into a pGEM7Zf$^+$ vector. SP6-driven transcription of the *Eco*RI-linearized construct yielded a 411-nucleotide riboprobe that upon hybridization was projected to generate a 354-nucleotide protected fragment as well as a 200-nucleotide protected fragment spanning a putative splicing variant previously reported for the human gene (38). A 385 *Xba*I-*Eco*RI fragment of the original PGS-2 cDNA was subcloned into a pGEM7Zf$^+$ vector. T7-driven transcription of the *Hin*dIII-linearized construct yielded a 328-nucleotide riboprobe that upon hybridization was projected to generate a 297-nucleotide protected fragment. The ribosomal protein large 19 (RPL19) probe was generated and employed as previously described (9).

RNA extraction

RNA of cultured cells and of tissues was extracted with RNAzol-B (Tel Test, Friendswood, TX) according to the manufacturer's protocol.

RNase protection assay

Linearized DNA templates were transcribed with the appropriate RNA polymerase to specific activities of 800 Ci/mmol [α-^{32}P]UTP (PGS-1 and PGS-2) and 160 Ci/mmol [α-^{32}P]UTP (RPL19). The riboprobes were gel purified as previously described (39) in an effort to eliminate transcribed products shorter than the full-length probes. The assay was performed as previously described (40). To generate quantitative data, gels were also exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA). The resultant digitized data were analyzed with ImageQuant Software (Molecular Dynamics, Sunnyvale, CA). The hormonally independent RPL19 messenger RNA (mRNA) signal was used to normalize the PGS-1 and PGS-2 mRNA data for possible variation in RNA loads. Specifically, the net protected signal (respective background subtracted) to net RPL19 ratio was calculated for each sample and gene of interest.

HPLC

Conditioned media were acidified to pH 3.5 with 3% formic acid and extracted twice with 3 ml ethyl acetate. Extracts were evaporated to dryness and reconstituted in 95:5 hexane-isopropyl alcohol for separation by normal phase HPLC on a silica gel column containing a chemically bonded diol phase (10 μm LiChrosorb Diol, EM Reagents, VWR Scientific, San Francisco, CA) with the use of a Waters/Millipore HPLC system (Milford, MA). The PG-containing extracts were applied to the column and eluted at 2 ml/min using a concave 50-ml gradient from 95:5 to 60:40 hexane-isopropyl alcohol. Radiolabeled arachidonic acid and its metabolites were detected and quantified in-line by liquid scintillation counting (Flo-One/Beta Radioactive Flow Detector, Packard Instruments Co., Downers Grove, IL).

Immune Western blot analysis

Methodology conformed to that previously described (19, 41, 42). Filters were incubated with affinity-purified antibody 9181, which recognizes both PGS-1 and PGS-2 (20, 42, 43), and with antibody 8223, which is selective for PGS-1 (20, 41, 42).

Data analysis

Except as noted, each experiment was replicated a minimum of three times. Data points are presented as the mean $\pm$ SE. Statistical significance was determined by ANOVA (Fisher's protected least significance difference) or Student's *t* test using Statview 512$^+$ for MacIntosh (Brain Power, Calabasas, CA).

Results

Ovarian PGS-1 and PGS-2 gene expression: effect of follicular maturation, ovulation, and corpus luteum formation

To assess PGS-1 and PGS-2 gene expression in the course of a simulated estrous cycle, 25-day-old rats were initially primed with PMSG (15 IU). Ovulation was triggered 48 h

later with hCG (15 IU). The animals were killed at the indicated time points, and total ovarian RNA was subjected to a solution hybridization RNase protection assay with antisense riboprobes corresponding to rat PGS-1, PGS-2, and RPL19. As shown (Fig. 1, *right panel*), protected fragments corresponding to PGS-1 and PGS-2 were apparent throughout the experiment. PGS-1 transcripts, in turn, were not significantly altered during the periovulatory period. Still, a 2.5-fold increase was documented. In contrast, the *in vivo* expression of PGS-2 rose substantially to a peak (35-fold increase; $P < 0.05$) 8 h after hCG administration. In one of three experiments (shown in Fig. 1), the PGS-2 peak was noted 12 h after hCG administration. A marked decrease to baseline was noted 24 h after hCG administration.

PGS transcripts, proteins, and activity expression by cultured whole ovarian dispersates: IL-1 dose and time dependence

Given the apparent *in vivo* ability of hCG to up-regulate both ovarian IL-1β (6) and PGS-2 transcripts (Fig. 1), we examined a possible *in vitro* effect of IL-1β on PGS-2 gene expression. Whole ovarian dispersates from immature rats were cultured for 48 h in the absence or presence of increasing concentrations (0.001–10 ng/ml) of IL-1β. As shown (Fig. 2), treatment produced dose-dependent increments in PGS-2

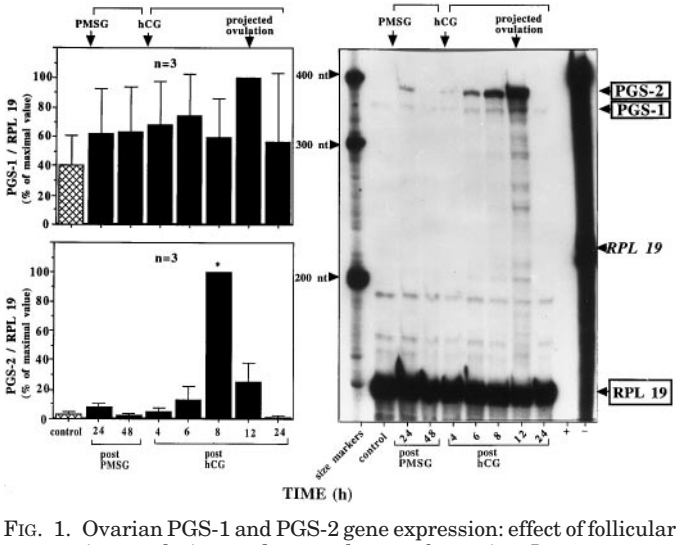

FIG. 1. Ovarian PGS-1 and PGS-2 gene expression: effect of follicular maturation, ovulation, and corpus luteum formation. Immature rats were initially primed with 15 IU PMSG. Ovulation was triggered 48 h later with 15 IU hCG. The animals were killed at the indicated time points, the ovaries were snap-frozen at −70 C, and total RNA (20 μg) was extracted and subjected to a solution hybridization RNase protection assay with antisense riboprobes corresponding to rat PGS-1, PGS-2, and RPL19. The intensity of the signals was quantified as described. *Left panels* depict (in bar graph form) the mean ± SE of three experiments. In each individual experiment data were normalized relative to the maximal value. The *right panel* depicts a representative autoradiograph. The *plus* and *minus symbols* designate riboprobe lanes treated with or without RNase, respectively. Protected fragments are depicted in *boldface letters*. Full-length riboprobes are depicted in *italics*. In this particular experiment, the PGS-2 construct was linearized with *Eco*RI, thereby producing a 421-bp probe and a 390-bp protected fragment. In this (one of three) experiment, the PGS-2 peak was noted 12 h (rather than 8 h) after hCG administration. *, $P < 0.05$.

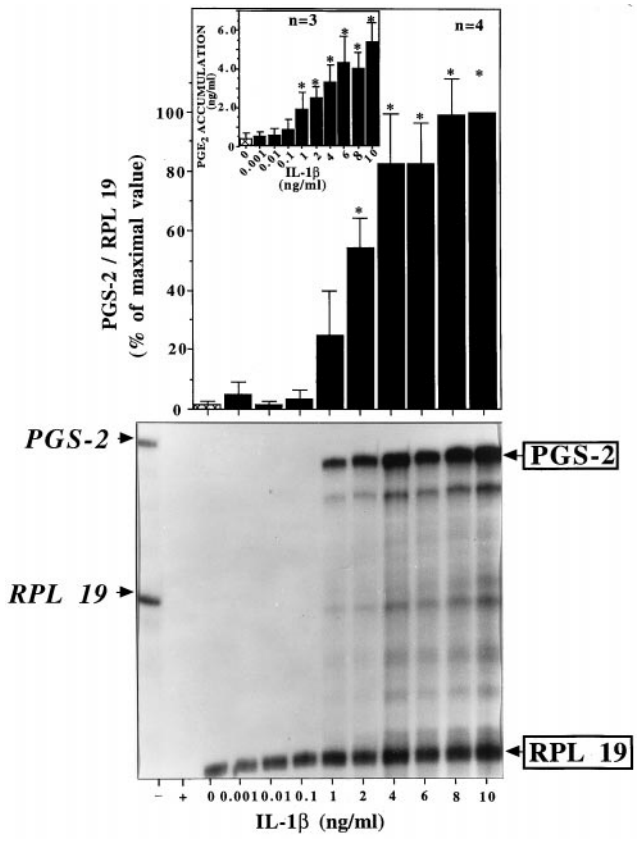

FIG. 2. PGS-2 gene expression by cultured whole ovarian dispersates: IL-1 dose dependence. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were cultured for 48 h in the absence or presence of increasing concentrations of IL-1β. Total cellular RNA was extracted and subjected to a RNase protection assay using antisense riboprobes corresponding to rat PGS-2 and RPL19. The intensity of the signals was quantified as described. The *upper panel* depicts (in bar graph form) the mean ± SE of four experiments. In each experiment, data were normalized relative to the maximal value. The *inset* depicts (in bar graph form) the mean ± SE of three experiments concerned with the accumulation of medium PGE_2. The *lower panel* depicts a representative autoradiograph. The *plus* and *minus symbols* designate riboprobe lanes treated with or without RNase, respectively. Protected fragments are labeled in *boldface letters*. Full-length riboprobes are labeled in *italics*. *, $P < 0.05$.

gene expression; the first significant ($P < 0.01$) increase was detected at the 1ng/ml dose level. The ability of IL-1β to up-regulate ovarian PGS-2 gene expression was characterized by an approximate ED_{50} of 2 ng/ml and a maximal response (at the 10 ng/ml dose level) representing a 72-fold increase over the untreated control value ($P < 0.001$). In contrast, treatment with IL-1β produced only a 3.4-fold increment ($P < 0.005$) in PGS-1 transcripts (Fig. 3). Qualitatively comparable results were obtained for the accumulation of PGE_2 (Fig. 2, *inset*), for which a 13-fold increase was noted. Moreover, treatment with IL-1 markedly enhanced the metabolism of radiolabeled arachidonic acid (Fig. 4). The PGS-mediated conversion of arachidonic acid to (mainly) PGE_2 was amplified at least 3-fold above control levels.

To assess the time requirements of the IL-1 effect, whole ovarian dispersates were cultured for the duration indicated (up to 48 h) in the absence or presence of IL-1β (10 ng/ml).

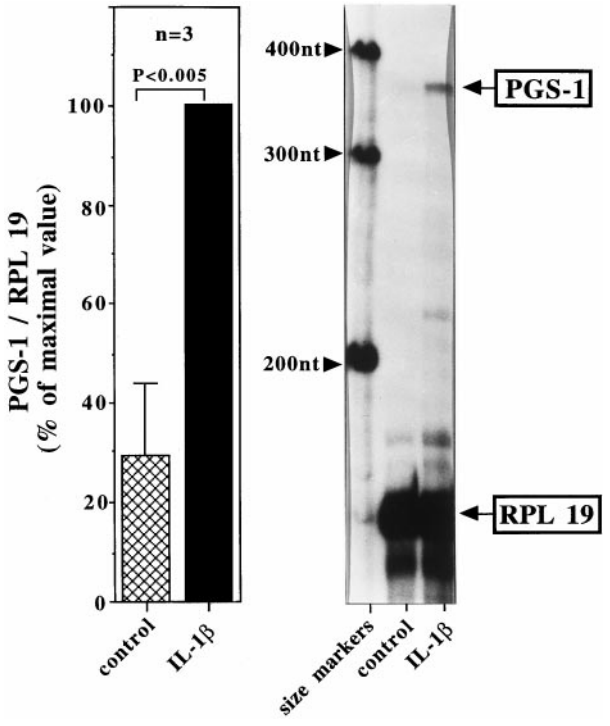

FIG. 3. PGS-1 gene expression by cultured whole ovarian dispersates: IL-1 dependence. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml). Total cellular RNA was extracted and subjected to a RNase protection assay using antisense riboprobes corresponding to rat PGS-1 and RPL19. The intensity of the signals was quantified as described. The *left panel* depicts (in bar graph form) the mean ± SE of three experiments. In each experiment, data were normalized relative to the maximal value. The *right panel* depicts a representative autoradiograph. Protected fragments are labeled in *boldface letters*.

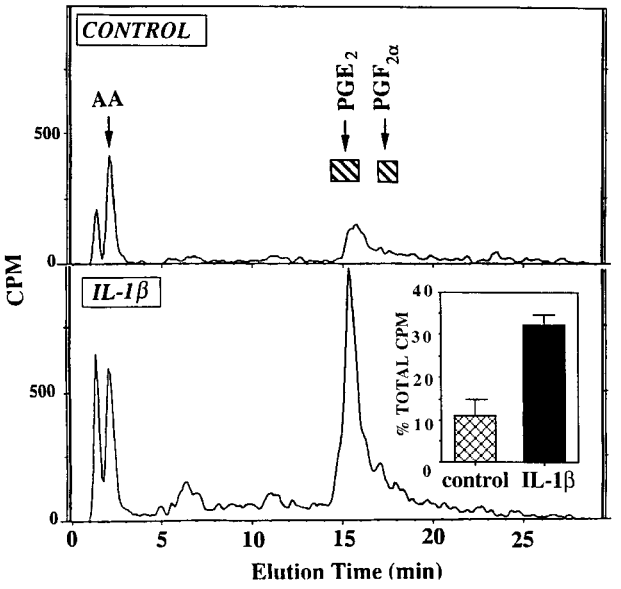

FIG. 4. PGS activity in cultured whole ovarian dispersates: IL-1 dependence. Whole ovarian dispersates (5×10^5 viable cells/dish) were cultured for 72 h in the absence (*top panel*) or presence (*bottom panel*) of IL-1β (50 ng/ml) and [^{3}H]arachidonic acid (AA; 20,000 cpm). At the conclusion of the incubation period, media were collected, and their PG constituents were separated by normal phase HPLC. The *hatched boxes* and *arrows* indicate the range of elution times for authentic standards. The *inset* (*bottom panel*) summarizes the quantities (mean ± SE) of substrate metabolized to PGE_2 (and $PGF_{2\alpha}$) in the absence or presence of IL-1β (two experiments; two or three replicates each).

As shown (Fig. 5), the IL-1β effect proved time dependent; the first significant ($P < 0.005$) increase in PGS-2 transcripts (over the untreated control value) was detected at 20 h, and the maximal response constituted a 43-fold increase in PGS-2 gene expression by 48 h of culture. Qualitatively comparable time-dependent effects were noted when assessing the immunoreactive content of PGS-2 (Fig. 6, *upper panel*) as determined by immune Western blot analysis carried out with the PGS-2-directed antibody 9181. In addition to the 72-kDa holoenzyme, a 59-kDa proteolytic fragment was noted, in keeping with previous observations (41, 42). In contrast, only a modest signal was apparent for the immunoreactive content of PGS-1, as assessed by immune Western blotting using PGS-1-specific antibody 8223 (Fig. 9, *lower panel*).

IL-1β-induced PGS-2 gene expression: receptor mediation

To explore the possibility that the IL-1β-induced PGS-2 gene expression constitutes a receptor-mediated event, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without human recombinant IL-1RA (1 μg/ml), a reagent previously validated at the ovarian level (43). As shown (Fig. 7), treatment with IL-1β produced a 27-fold increase in the relative expression of PGS-2 transcripts over the untreated control value ($P < 0.001$). In contrast, treatment with IL-1RA by itself had no significant effect. However, the concurrent provision of IL-1RA resulted in substantial ($P < 0.001$) attenuation of the IL-1β effect to a level indistinguishable from that displayed by untreated control counterparts. These observations support the suggestion that the ability of IL-1β to upregulate PGS-2 transcripts is IL-1 receptor mediated. Qualitatively comparable results were documented at the level of immunoreactive PGS-2, as assessed by immune Western blot analysis (Fig. 8, *lower panel*). A comparable phenomenon was noted for the ability of IL-1β to stimulate (6.1-fold increase; $P < 0.05$) the accumulation of medium PGE_2 (Fig. 8, *upper panel*), which was also receptor mediated.

IL-1β-induced PGS-2 gene expression: role of cell-cell cooperation

Whole ovarian dispersates constitute a heterogeneous mix of multiple ovarian cell types (predominantly granulosa and thecal-interstitial cells at a ratio of 4:1). IL-1β-stimulated PG biosynthesis proved contingent upon heterologous contact-independent cell-cell interaction (30). We, therefore, assessed the effect of cell-cell cooperation on PGS expression. Cells were cultured on standard plastic substratum under serum-free conditions for 96 h in the absence or presence of IL-1β. As shown (Fig. 9), treatment of isolated granulosa cells (5×10^5 viable cells/dish) with IL-1β proved only minimally effective in altering their immunoreactive PGS-2 content (or PGS-2 transcripts; not shown). Similarly, treatment with IL-1β was without significant effect on isolated, highly purified thecal-interstitial cells (Fig. 9). However, cell contact-

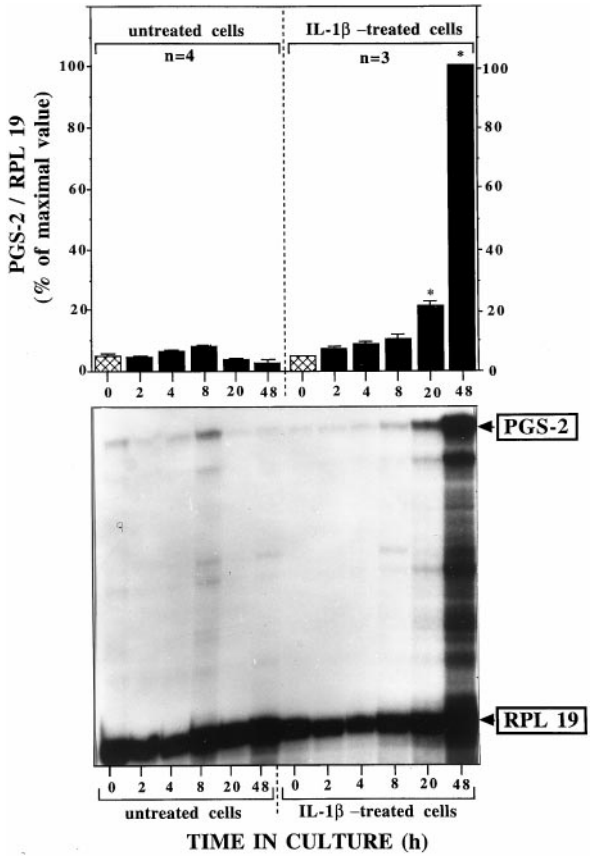

FIG. 5. PGS-2 gene expression by cultured whole ovarian dispersates: IL-1 time dependence. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were cultured for the duration indicated (up to 48 h) in the absence or presence of IL-1β (10 ng/ml). Total cellular RNA was extracted and subjected to a RNase protection assay using antisense riboprobes corresponding to rat PGS-2 and RPL19. The intensity of the signals was quantified as described. *Upper panels* depict (in bar graph form) the mean ± SE of three or four experiments. In each experiment, data were normalized relative to the peak IL-1β value. The *lower panel* depicts a representative autoradiograph. Protected fragments are labeled in *boldface letters*. *, $P < 0.005$ *vs.* time zero.

dependent cocultures of isolated granulosa (4×10^5 cells/dish) and highly purified thecal-interstitial (1×10^5 cells/dish) cells (4:1 ratio) restored IL-1 responsiveness to a level comparable to that noted for whole ovarian dispersates (Fig. 9). These findings suggest that IL-1 action is contingent upon heterologous cell-cell interaction.

IL-1β-induced PGS-2 gene expression by cultured whole ovarian dispersates: effect of granulosa cell agonists

To determine the effect of ovarian agonists other than IL-1β, whole ovarian dispersates were cultured for 48 h in the absence or presence of IGF-I, activin-A, ET-1, TGFα, or IL-1β. As shown (Fig. 10), none of the above-mentioned agonists (with the exception of IL-1β) affected PGS-2 gene expression compared with untreated cells. Similarly, a series of representative cytokines, including TNFα, vascular endothelial growth factor, leukemia inhibitor factor, hepatocyte growth factor, and keratinocyte growth factor, proved without significant effect on PGS-2 gene expression (not shown).

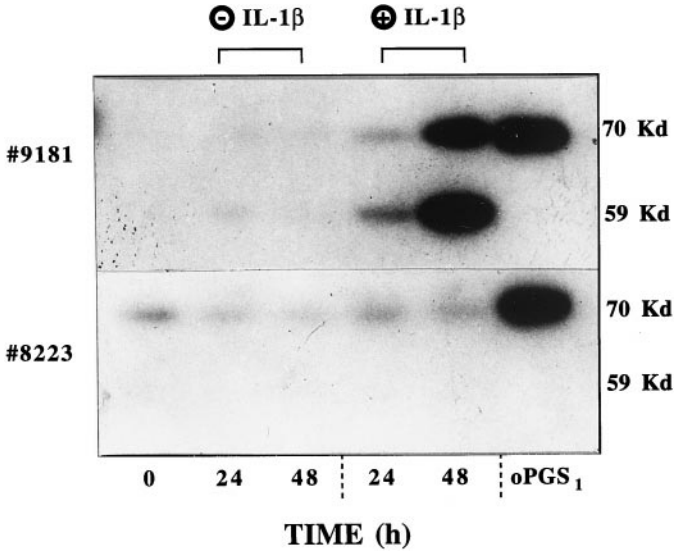

FIG. 6. IL-1-induced PGS-2 protein: IL-1 and time dependence. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were cultured for the duration indicated (up to 48 h) in the absence or presence of IL-1β (50 ng/ml). The immunoreactive contents of PGS-1 (antibody 8223) and PGS-2 (antibody 9181) were determined by immune Western blot analysis (50 μg protein/lane). In addition to the 72-kDa holoenzyme, a 59-kDa proteolytic fragment was noted, in keeping with previous observations (41, 42). $oPGS_1$, Ovine PGS-1 standard (12.5 ng/lane).

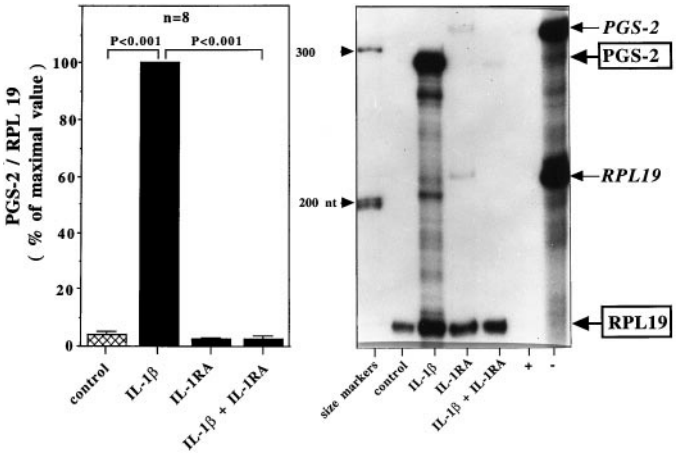

FIG. 7. IL-1-induced PGS-2 gene expression: receptor mediation. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without human recombinant IL-1 RA (1 μg/ml). Medium PGE_2 content was measured by RIA. The resultant RNA samples were subjected to a RNase protection assay using antisense riboprobes corresponding to rat PGS-2 and RPL19. The intensity of the signals was quantified as described. The *left panel* depicts (in bar graph form) the mean ± SE of eight experiments. In each experiment, data were normalized relative to the IL-1β peak value. The *right panel* depicts a representative autoradiograph. The *plus* and *minus symbols* designate riboprobe lanes treated with or without RNase, respectively. Protected fragments are labeled in *boldface letters*. Full-length riboprobes are labeled in *italics*.

Discussion

Our present findings document, for the first time, that no significant changes are noted for PGS-1 during a simulated estrous cycle. In contrast, marked periovulatory increments

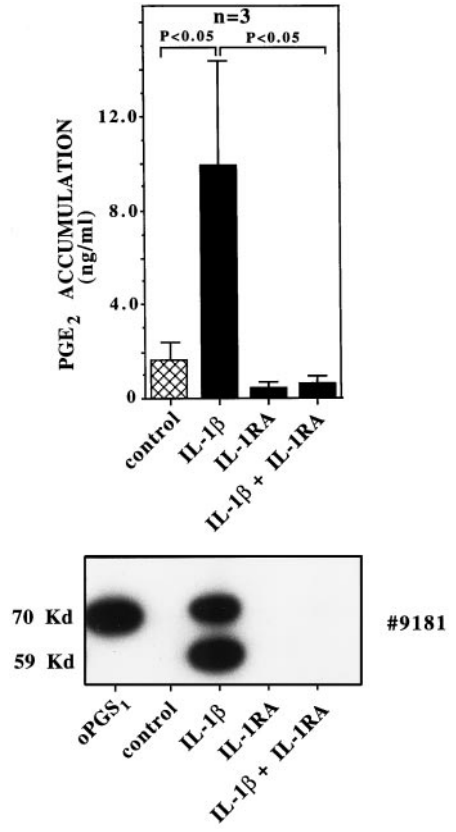

FIG. 8. IL-1β-induced PGS-2 protein and PGE_2 biosynthesis: receptor mediation. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without human recombinant IL-1RA (1 μg/ml). Medium PGE_2 content was measured by RIA. The immunoreactive content of PGS-2 was determined by immune Western blot analysis (antibody 9181). In addition to the 72-kDa holoenzyme, a 59-kDa fragment was noted, in keeping with previous observations (41, 42). The *upper panel* depicts (in bar graph form) the mean ± SE of three experiments. The *lower panel* depicts a representative autoradiograph. $oPGS_1$, Ovine PGS-1 standard (12.5 ng/lane).

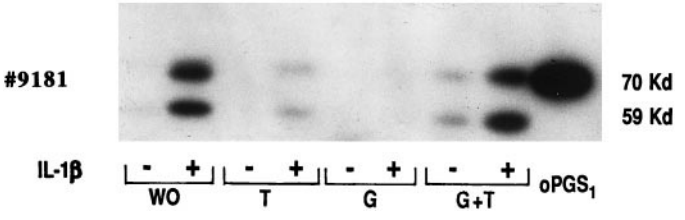

FIG. 9. IL-1-induced PGS-2 protein in cultured ovarian cells: role of cell-cell cooperation. Isolated granulosa (G) cells (5×10^5 viable cells/dish); isolated, highly purified, thecal-interstitial (T) cells (1×10^5 viable cells/dish); contact-dependent cocultures thereof (G+T); or whole ovarian dispersates (WO) were cultured under serum-free conditions for 96 h in the absence or presence of IL-1β. The immunoreactive content of PGS-2 was determined by immune Western blot analysis (antibody 9181). In addition to the 72-kDa holoenzyme, a 59-kDa fragment was noted, in keeping with previous observations (41, 42). $oPGS_1$, Ovine PGS-1 standard (12.5 ng/lane).

were noted for PGS-2 transcripts approximately 8–12 h after hCG administration. Given that ovulation is projected to occur 12 h after hCG administration, these findings suggest peak ovarian PGS-2 expression just before or during ovulation. Similar findings were reported for PGS-2 mRNA and protein, which were regulated by hCG in bovine (20) and rat (44) preovulatory follicles. Accordingly, temporal considerations alone would suggest that PGS-2 (but not PGS-1) may play a role during the periovulatory period and, by extension, in the process of follicular rupture.

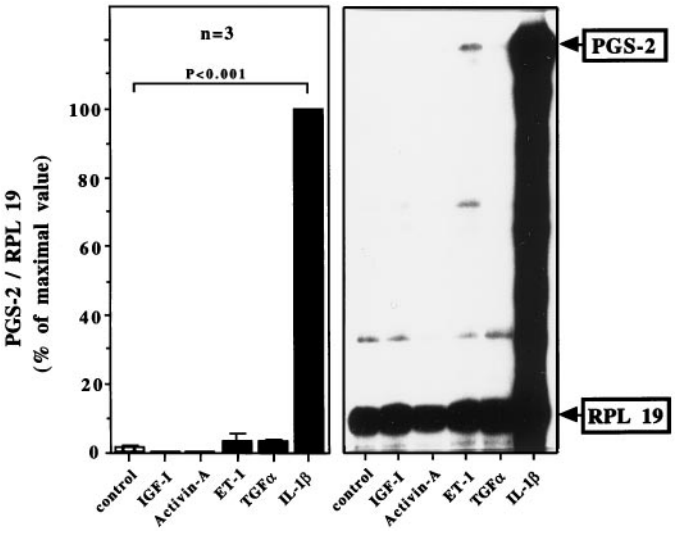

FIG. 10. IL-1β-induced PGS-2 gene expression: specificity studies. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were cultured for 48 h in the absence or presence of IGF-I (50 ng/ml), activin A (50 ng/ml), ET-1 (10^{-7} M)), TGFα (10 ng/ml), or IL-1β (10 ng/ml). Total cellular RNA was extracted and subjected to a RNase protection assay, using antisense riboprobes corresponding to rat PGS-2 and RPL19. The intensity of the signals was quantified as described. The *left panel* depicts (in bar graph form) the mean ± SE of three experiments. In each experiment, data were normalized relative to the IL-1β peak value. The *right panel* depicts a representative autoradiograph. Protected fragments are depicted in *boldface letters*.

Previous studies have clearly established the preovulatory rat granulosa cell as the main site of immunoreactive PGS expression (19, 41, 45). Sirois and associates localized the PGS-2 protein to the granulosa cell (42). Wong and associates, in turn, observed immunoreactive PGS-1 in the thecal cell layer (41). Modest immunoreactive PGS expression beyond the follicular basement membrane has also been documented (19, 45). Consequently, one must assume that the detection of relevant transcripts in whole ovarian material (Fig. 1) or in cultured whole ovarian dispersates (Figs. 2, 3, 7, and 10) of rat origin depends on the contribution of multiple cellular compartments inclusive of granulosa and thecal cell elements.

Although the dependence of the PGS-2 gene on IL-1β has been demonstrated in several extraovarian sites (46–48), the ability of IL-1β to modulate ovarian PGS gene expression has received limited attention. In fact, Sirois and associates failed to document a stimulatory effect of IL-1β on PGS-2 expression in primary cultures of rat granulosa cells, as assessed by the induction of chloramphenicol acetyltransferase reporter gene activity (49). It appears highly likely that the apparent inability of IL-1 to stimulate the PGS-2 promoter reflects the limited responsiveness of the isolated granulosa cell to this agonist and the apparent obligatory dependence on heterologous cell-cell cooperation (30) (Fig. 9). More puzzling is

the observation of Wong and Richards (50) about the apparent inability of IL-1β to induce PGS protein in cultured preovulatory follicles exposed for a total of 7 h. Although the above argues against the projected dependence on cell-cell cooperation, consideration must be given to the possibility that the short term exposure precludes unequivocal conclusions in this regard.

We herein document the ability of IL-1β to produce dose- and time-dependent increments in PGS-2 gene expression, as assessed in cultured whole ovarian dispersates from immature rats. Qualitatively comparable up-regulation of PGS-2 protein (Figs. 6, 8, and 9) and PGS activity (Fig. 4) were also noted. Although treatment with IL-1β led to an increase in PGS-1 transcripts as well as in immunoreactive PGS-1 protein, the relative magnitude of the effect was markedly reduced compared with that noted for PGS-2. However, we cannot rule out modest cross-reactivity of the PGS-1-directed antibody with the abundant PGS-2 protein.

Importantly, the IL-1 effect appeared to be receptor mediated, in that IL-1β action was completely abrogated in the presence of IL-1RA. It is highly likely the IL-1 effect is mediated via the type I IL-1 receptor, the role of which in signal transduction has been amply documented (51). Indeed, the type II IL-1 receptor may be an IL-1-binding protein or decoy receptor (52), the overall abundance of which in the rat ovary is substantially reduced (9).

Studies at the transcript levels suggest that the IL-1 effect is characterized by an approximate ED_{50} of 2 ng/ml. This ED_{50} is higher than that required for the induction of secretory PLA_2 transcripts (0.3 ng/ml) or nitric oxide synthase activity (0.7 ng/ml), comparable to that required for the induction of GLUT1 and GLUT3 transcripts (2.0 and 3.0 ng/ml, respectively) or cytosolic phospholipase A_2 (PLA_2; 2 ng/ml), but lower than that required for the induction of IL-1β (6 ng/ml) or type IL-1 receptor (10 ng/ml) transcripts (53, 54) (Kol, S., K. Ruutiainen-Altman, W. J. Scherzer, I. Ben-Shlomo, M. Ando, R. M. Rohan, and E. Y. Adashi, unpublished observations). To the extent that IL-1 may play a role in the ovulatory cascade (1), these observations suggest that the induction of PGS-2 may constitute one of the early and most sensitive events in the sequence leading to follicular rupture.

Although our present findings reveal IL-1 as an inducer of PGS transcription and activity, the role and identity of the cells affected remain to be established. Intuitive reasoning alone would suggest that most, if not all, of the cells targeted are granulosa cells, as this cell type comprises 80% of the total ovarian cellular population (55). Moreover, the granulosa cell is the main site of PGS-2 expression (19, 45). However, our current findings reveal IL-1 to be without significant effect on the isolated granulosa cell. It is, therefore, tempting to speculate that the action of IL-1 at the level of the granulosa cell requires the concurrent presence of other ovarian cellular components. That this, in fact, is the case is attested to by our current finding that the addition of highly purified thecal-interstitial cells to isolated granulosa cell preparations reestablishes IL-1 responsiveness. Taken together, these observations confirm previous ones that the action of IL-1 is obligatorily dependent on cell-cell cooperation representative of two distinct ovarian compartments (10, 11, 15, 56). Reasoning along these lines further suggests that IL-1 may not be the ultimate effector, but that it may be in a position to induce the cooperative elaboration of a soluble principal to serve in this role.

This laboratory has previously demonstrated the ability of IL-1 to stimulate the biosynthesis of PG in cultured whole ovarian dispersates (30). We have since been able to document that this PG-promoting property of IL-1 is due in part to the up-regulation of ovarian secretory PLA_2 and cytosolic PLA_2 (53). In this communication, it is documented that the PG-promoting property of IL-1 is also due in large measure to the induction of PGS-2. Consequently, the ability of IL-1 to promote ovarian PG biosynthesis involves the activation of several enzymatic steps along the biosynthetic cascade. Future studies will focus on the potential role of IL-1 in up-regulating PLA_2-activating protein, PG transport, and/or PG receptors.

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